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GEO. T. WINSTON, LL.D., DIRECTOR

The Composition of Cotton-seed Meal

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WEST RALEIGH, N. C.
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THE NORTH CAROLINA COLLEGE OF AGRICULTURE AND MECHANIC ARTS

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The Composition of Cottonseed Meal.

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Most of the analyses of cottonseed meal consist merely of determinations of the fodder groups—*i. e.*, water, fat protein, nitrogen-free extract, crude fiber, and ash. We have undertaken to determine the quantity of some of the proximate constituents, and in order to obtain average values, have used a number of samples of meal.

The meals used were obtained from oil mills in different sections (we desire to express our thanks for their kindness in sending them), and, as might be expected, were above the average as regards protein content and crude fiber. Their description is given in the following table (Table I)):

TABLE I.—*Description of Samples.*

Number	Source.	
1414	Unknown*	
1551	Southern Cotton Oil Co	Memphis, Tenn.
1553	Charlotte Oil and Fertilizer Co	Charlotte, N. C.
1554	N. C. Cotton Oil Co	Charlotte, N. C.
1555	Southern Cotton Oil Co. (Sea Island cottonseed meal)	Savannah, Ga.
1556	Goldsboro Oil Co†	Goldsboro, N. C.
1557	Tar River Oil Co	Tarboro, N. C.
1593	N. C. Cotton Oil Co	Wilmington, N.C.
1604	Edgecombe County Cotton Oil Co	Conetoe, N. C.
1611	Unknown*	

* Probably from N. C. Cotton Oil Mills, Raleigh.
† From lot bought by them.

FODDER GROUPS.

In making the regular feeding-stuff analyses, the methods of the Association of Official Agricultural Chemists were used [Bulletin 46, Revised Edition, Division of Chemistry, U. S. Department of Agriculture (1899)]. The following method of filtration was found very satisfactory in the crude fiber determination: A very thin filter of ignited asbestos is made in a three-inch Hirsch funnel and a perforated disk of platinum

laid on it to prevent it from being destroyed when the liquid is poured on. When the digestion with dilute sulphuric acid is completed, the particles are allowed to subside before filtering, if the substance is cottonseed meal, cowpea meal, or similar substances; but if it is a coarse fodder, or excrement, the liquid is shaken well just before it is poured on the filter. The filtration takes place very rapidly under these conditions. The asbestos and organic matter, after being washed, are transferred back into the flask with boiling alkali. The second filtration is made on a similar filter, and the contents of the dish, after being washed, are transferred to a platinum dish, dried, etc., as usual.

The results of the feeding-stuff analyses are given in the fol lowing table (Table II):

TABLE II.—*Fodder Analysis of Meals.*

Number.	Water.	Ash.	Protein.	Ether Extract.	N-Free Extract.	Crude Fiber.
1551	8.00	6.58	45.94	13.74	21.25	4.49
1553	8.77	6.43	44.85	9.48	25.16	5.31
1554	8.34	5.82	47.44	9.75	23.03	5.62
1556	7.80	6.12	48.50	10.86	21.29	5.43
1557	8.65	7.44	43.72	9.64	25.73	4.82
1593	8.12	6.24	45.18	7.88	23.86	8.72
1611	6.68	6.81	46.44	9.62	25.71	4.74
1604	6.70	6.98	49.37	10.24	20.96	5.76
1414	10.70	6.44	42.91	11.56	21.53	6.86
Maximum	10.70	7.44	49.37	13.74	25.71	8.72
Minimum	6.68	5.82	42.91	7.88	20.96	4.49
Average	8.20	6.54	46.04	10.30	23.17	5.75
Average of over 400	8.52	7.02	43.26	13.45	22.31	5.44
1555 (S. I.)	9.70	4.46	24.67	6.33	34.16	20.68
Average undecor.	11.64	6.26	24.08	5.91	31.43	20.68

The average of the nine analyses by us is compared with the average of 400 analyses [The Cotton Plant, Bulletin 33, Office of Experiment Stations, U. S. Department of Agriculture (1896)]. The average for protein is somewhat higher, and for fat, lower. The average of the 400 determinations in the case of fat (13.45 per cent) is too high now, since improvements in the methods of extraction have been made. The average amount of fat found in 205 analyses of cottonseed meal made in the New England States in 1898 and 1899 is 11.2 per cent (Conn. Station Bulletin No. 130).

No. 1555, as already stated, is a Sea Island cottonseed meal.

The meals bearing this name now on the market seem to be made from undecorticated seed, and are low in protein, and high in crude fiber. The analysis of 1555 is compared with the average of 62 analyses of undecorticated cottonseed cake (The Cotton Plant, p. 101), and the agreement is remarkably close.

BETAIN AND CHOLIN.

Ritthausen and Weger [Jour. Prakt. Chem. *30*, 32 (1884)] found betain in cottonseed meal, and cholin was separated by Boehm. Maxwell [Am. Chem. J. *13*, 469 (1891)] separated cholin and betain from cottonseed meal, and found them to be present in the following proportions: Cholin 17.5 per cent, betain 82.5 per cent. The method used by him was as follows: About five pounds of meal were extracted with 70 per cent alcohol, alcohol being used instead of dilute mineral acid to prevent any possibility of conversion of cholin to betain. The alcohol was distilled from the extract, and the residue taken up in water. The solution was treated with lead acetate, filtered, the excess of lead removed from the filtrate, and the latter evaporated to a syrupy residue. The residue was extracted with 70 per cent alcohol containing 1 per cent hydrochloric acid, and the alkaloids precipitated with alcoholic mercuric chlorid. After standing ten days, the precipitate was separated, and the mother liquor put aside for some weeks, when a second crop of crystals was obtained. The double salts were recrystallized from water, decomposed with hydrogen sulphid, filtered, and the filtrate slowly evaporated to a small volume. It was then placed in a desiccator over sulphuric acid until the crystallization of the salts was complete, and all moisture was absorbed. The residue was then treated with absolute alcohol, which dissolves the cholin hydrochlorid, and dissolves only traces of the betain hydrochlorid, the solution of cholin hydrochlorid was evaported to dryness, and re-extracted with absolute alcohol three times, and the two products weighed. Maxwell's work was not a quantitative determination, but he obtained from about five pounds of cottonseed meal (2270 gram.), at least 7 grams of the mixed chlorids, or about 0.24 per cent of cholin and betain.

For the quantitative determination of betain and cholin, precipitation with alcoholic mercuric chlorid in alcoholic solution did not appear to us to be promising. When 100 grams of meal were used, and the solution (which had been purified, and evaporated to a small volume) treated with mercuric chlorid, and allowed to stand two weeks, only a very small precipitate separated. The following method was used: It gives approxi-

mate results. 150 grams of meal were boiled with 1200 cc of 75 per cent alcohol containing 1 cc of concentrated hydrochloric acid, filtered, an aliquot portion of the filtrate taken, and the alcohol distilled off The residue was treated with an excess of lead acetate, and filtered. The lead in the filtrate was precipitated with sulphuric acid, and filtered off, and the betain and cholin precipitated with phospho-tungstic acid. After standing two or more days, the precipitate was filtered off, and kjeldahled, the betain and cholin being calculated from the nitrogen found. The results are given below. It must be remembered that the method gives only approximate results.

		Per Cent.
1534	Betain and Cholin	0.47
1551	" "	0.26
1553	" "	0.16
1554	" "	0.25
1555	" "	0.20
1556	" "	0.27
1557	" "	0.26
	Average	0.28

The relative proportions of betain and cholin were determined as follows: The phospho-tungstic acid precipitate obtained as described above was treated with cold milk of lime, allowed to stand some hours, and filtered. The lime in the filtrate was precipitated with carbon dioxide, the vessel being allowed to stand over night before filtering, so that any excess of carbon dioxid might escape. The liquid was then filtered, made acid with hydrochloric acid, evaporated nearly to dryness, treated with alcohol, and after the evaporation of the alcohol allowed to stand several days in a desiccator over sulphuric acid to remove the last traces of moisture. It was then extracted with cold absolute alcohol. Nitrogen was determined in the extract (containing betain hydrochlorid), and also in the residue (cholin hydrochlorid), by the kjeldahl method.

	1534	1551
Betain, per cent	0.37	0.20
Cholin, per cent	0.10	0.055
Betain : Cholin	79 : 21	78 : 22
Betain (Maxwell)	82.5 : 17.5	

GOSSYPEIN.

F. Werenskiold (Expt. St. Record *9*, 805, abs.), by shaking the acid extract with chloroform, separated from cottonseed meal, an alkaloid that gives the same reactions as cholin and betain with iodin-potassium iodid, phospho-tungstic acid, picric acid,

and some other reagents, but different reactions with gold chlorid and platinum chlorid. He proposed the name "gossypein" for this alkaloid.

Gossypein, if present in the sample tested, was present in very minute quantity. The filtrate from 363 grams cottonseed meal, ready for precipitation with phospho-tungstic acid, was extracted with chloroform, and nitrogen was determined in the extract. It was equivalent to 0.008 per cent gossypein (calculated as cholin).

GOSSYPOL.

Marchlewski (Jahresber. f. Agr. Che. 1899, 241) found in cottonseed oil a phenol-like body, which had the formula $C_{25}H_{36}O_4$, and which he named gossypol. It is extracted from the oil with caustic soda, precipitated with hydrochloric acid, extracted from the precipitate with ether, and purified by crystallization from a mixture of alcohol and 50 per cent acetic acid.

PENTOSANS.

The method used for the determination of pentosans is that described in Bulletin No. 172 of this Station. The determinations are tabulated in Table III. A summary is given below:

	In Meal.	In N-free Extract.
Maximum, per cent	7.37	32.5
Minimum, per cent	6.32	25.3
Average (9 samples)	6.76	29.2
Sea Island meal	16.16	41.2

Very nearly 30 per cent of the nitrogen-free extract of cottonseed meal is composed of pentosans. The hulls are much richer in pentosans than the kernels; and the Sea Island cottonseed meal (from undecorticated seed) contains more than twice the quantity of pentosans found in other meal. The quantity of pentosans present will depend to a large extent upon the amount of hulls left in the meal.

Pentosans dissolved by disastase. As preliminary to the determination of starch (which was not present), determinations of diastase-soluble pentosans were made. The meal was treated with diastase solution as for the determination of starch (Methods of A.O.A.C.) and pentosans determined in a portion of the filtrate which had been evaporated to dryness The weights of the precipitate from 2.4 grams substance, after subtraction of the small quantity due to the diastase, were:

Sample I	0.00157 gm.
Sample II	0.0208 gm.
Sample III	0.0193 gm.

According to the methods in present use, these weights indicate the absence of pentosans.

Pentosans in crude fiber. Determinations of the pentosans in the crude fiber from the cottonseed meal showed that the crude fiber does not contain appreciable amounts of pentosans unless the meal contains a much larger quantity of hulls than is usually the case. The weights of the phloroglucid precipitates from the crude fiber from 3 grams material were, mean values:

No. 1553, 0.0202 grams; No. 1557, 0.0176 grams; No. 1555, 0.0797 grams.

As has already been stated, No. 1555 is meal from undecorticated cake. It contains:

	Per Cent.
Pentosans in nitrogen-free extract	14.07
Pentosans in crude fiber	2.09
Crude fiber in meal	20.68
Crude fiber contains pentosans	10.

The pentosans of the cottonseed hulls, like the pentosoids in many coarse fodders, are only partly insoluble in the reagents used for determining crude fiber.

STARCH.

Quantitative tests for starch were made by the diastase method in several of the samples; and none was found. No evidence of any traces of starch was detected by microscopic examination of seven of the samples of meal.

SUGARS.

The sugar of cottonseed meal is raffinose, a triose sugar, one molecule of which can be hydrolized to three molecules of a simple sugar.

Ritthausen [J. Prakt. Chem. *29*, 351 (1884)] separated a sugar from cottonseed, as also did Boehm [Ibid. *30*, 37 (1884)], which they believed identical with a sugar obtained by Berthelot from eucalyptus manna. They called it gossypose.

Scheibler [Ber. d. Chem. Ges. *18*, 1779 (1884)] studied the properties of raffinose from beet sugar and of gossypose, and found them to be identical

Rieschbiet and Tollens [Ber. d. Chem. Ges. *18*, 2611 (1885)] also made a study of the properties of raffinose, and they likewise found it to be identical with the gossypose of Boehm and Ritthausen.

Berthelot [Compt Rend. *103*, 533 (1886)] concluded from his investigations that the sugar from cottonseed meal was a combination of raffinose with an unfermentable substance, to which he gave the name eucalyn.

Scheibler and Mittelmeier [Ber. d. Chem. Ges. *22*, 3118 (1889)] showed that Berthelot's conclusions were due to an erroneous interpretation of facts, and that the substance to which he gave the name eucalyn was formed by the partial hydrolysis of raffinose.

Raffinose is composed of three molecules of sugars:

fructose-glucose-galactose.

When it is treated with hydrochloric acid under the same conditions as for the inversion of sucrose, it breaks down into two molecules of reducing sugars (Ber. d. Chem. Ges. *22*, 1680).

$$C_{18}H_{32}O_{16} + H_2O = C_{12}H_{22}O_{11} + C_6H_{12}O_6$$

If the inversion were carried further, three molecules of reducing sugars would be formed.

The quantity of raffinose may be found gravimetrically by determining as if it were sucrose, and multiplying the sucrose found by 1.5. The results would be too high if sucrose were present.

The reducing sugars and sucrose were determined in the cottonseed meal according to the methods of the A.O.A.C., with the use of six grams of material instead of three, and the further modification that the cuprous oxid was weighed directly, as recommended by Bartlett. The sucrose was then calculated to raffinose. The copper precipitate from the reducing sugars did not seem to be cuprous oxid, being flocculent in nature, and hence probably no reducing sugars are present.

The reducing sugars and raffinose found are given in the following table. (Table III).

TABLE III.—*Pentosans and Sugars.*

Number.	In Meal, Per Cent.			In N-Free Extract, Per Cent.		
	Red. Sugars.	Raffinose.	Pentosans.	Pentosans.	Sugars.	Residue.
1414	Trace	10.63	6.99	32.5	49.4	18.1
1551	0.22	9.72	6.36	29.9	46.8	23.3
1553	0.44	10.57	7.37	29.3	43.7	27.0
1554	0.34	10.23	7.18	31.2	45.9	22.9
1556	0.20	10.00	6.50	30.5	47.9	21.6
1557	0.02	10.48	6.56	25.5	40.9	33.6
1593	0.02	11.24	7.07	29.6	47.2	28.2
1604	0.15	11.50	6.32	30.1	55.6	14.3
1611	0.35	12.21	6.50	25.3	48.8	25.9
Maximum	0.44	12.21	7.37	32.5	55.6	33.6
Minimum	0.02	9.72	6.32	25.3	40.9	14.3
Average	0.22	10.73	6.76	29.2	47.4	23.3
1555 (S. I.)	0.10	5.73	16.16	41.2	17.1	41.2

Raffinose composes very nearly 50 per cent of the nitrogen-free extract, and sugars and pentosans together make up 76.7 per cent of it, on an average.

The New York Experiment Station (Geneva) in Bulletin 176 reports the results of determinations of starch and sugar in a number of feeding stuffs, including cottonseed meal. The starch and sugar were determined together by treating the material with diastase, then with hydrochloric acid, and then with copper solution, etc. A correction was made for the diastase. Their results were on cottonseed meal, eight samples:

	Sugars.	Sugars in N-free Extract.
Maximum, per cent	24.7	71.
Minimum	12.7	48.
Average	14.9	57.4

These figures are considerably higher than those we obtained for raffinose.

In a preceding Bulletin of this Station (No. 172) it was shown that, as a rule, sugars are completely digested. The digestibility of the pentosans in a number of materials given in that bulletin, is from 48 to 95 per cent. If all the sugars of cottonseed meal are digested and 50 per cent of the pentosans, the nitrogen-free extract should have a digestibility of 62 per cent, without considering the other constituents.

IS SUCROSE PRESENT?

It has been stated that the method of calculating raffinose would give too high results if sucrose were present. Some experiments were made to see if the meal contained sucrose in sufficient quantity to affect the results.

Five hundred grams of meal were stirred with five liters of water, and filtered after standing an hour or longer. The filtrate was then partly evaporated, lead acetate added in excess, and the precipitate filtered off. The filtrate was decolorized with bone black, and polarized. It was then inverted, and polarized again. The ratio of sucrose to raffinose was calculated in the following way:

$$Z \text{ (sucrose)} = \frac{S - O.493P}{O.827}$$

$$R \text{ (raffinose)} = \frac{P - Z}{1.57}$$

S is the algebraic difference between the direct polarization

(P) and the polarization after inversion. The ratio R: Z was found to be:

	Raffinose.		Sucrose.
1553	81	:	19
1556	86	:	14
1593	95	:	5
1551	100		
1557	100		

The solutions from Nos. 1551, 1553 and 1556 were tested for reducing sugars, but none were found. The reducing substances had been precipitated with lead acetate. This confirms the hypothesis that cottonseed meal does not contain reducing sugars.

As it was difficult to see why sucrose if present in cottonseed meal should vary so much from sample to sample, further tests of its presence were made. Other rotatory substances might be present and cause incorrect results.

The solution from sample number 1553, which was supposed to contain 81 parts of raffinose to 19 parts of sucrose was evaporated to 150 cc after removing lead with sodium chlorid. It was then saturated with barium hydroxide, allowed to stand for some time protected from the air, and then filtered. The precipitate was then mixed with water, the barium removed with carbon dioxide, and sucrose and raffinose determined in it with the polariscope No sucrose was found. The filtrate was mixed with alcohol, which should precipitate barium sucrate, if any were present, together with barium raffinate. The precipitate was dissolved in water, and raffinose determined in it as before. No sucrose was found. We may conclude from this experiment that cottonseed meal does not contain sucrose in appreciable quantity. The method is not delicate enough to detect small quantities.

ORGANIC ACIDS.

Organic acids were determined in the following way: 25 grams of substance were mixed with water containing 10 cc of hydrochloric acid 1:10, and allowed to stand an hour. The solution was filtered, and the residue washed with water. A sufficient quantity of a 5 per cent solution of caustic potash was added to neutralize the acid used, the organic acids in the solution were precipitated with lead subacetate, and filtered, and the precipitate was washed. The latter was mixed with water, decomposed hot with hydrogen sulphid, filtered and washed. The filtrate was evaporated to about 50 cc, neutralized with caustic potash, calcium chlorid and alcohol were added, and the mixture allowed to stand two days. The pre-

cipitate was then filtered off, washed with alcohol, ignited, dissolved in fifth normal HCl, and the excess titrated with tenth normal ammonia. The results were calculated as tartaric acid, and were as follows:

Number.	Per Cent.
1551	0.30
1553	0.45
1554	0.52
1555	0.67
1557	0.48
Mean	0.48

WATER-SOLUBLE MATTER.

The water-soluble constituents of the meal were determined as follows: 3 grams were extracted with ether, then stirred for some time with 50 cc water, filtered, and washed to 200 cc, 50 cc of the filtrate were evaporated to dryness in a platinum dish, and ignited. Another portion of 50 cc was used for the nitrogen determination. The water-soluble constituents of one sample were:

	Per Cent.
Sugars	10.57
Nitrogenous matter	5.55
Acids	0.52
Residue	2.25
Ash	3.83

The 2.25 per cent of water-soluble organic matter not accounted for is nearly 10 per cent of the total nitrogen-free extract, and about 43 per cent of the 22.9 per cent of the nitrogen-free extract unaccounted for.

SUMMARY.

The average amount of betain and cholin in seven samples of cottonseed meal is 0.28 per cent. The ratio betain: cholin in two samples is 79:21 and 78:22.

Gossypein, if present, is in minute quantity.

Of the nitrogen-free extract in nine samples, 29.2 per cent is composed of pentosans, and 47.4 per cent of raffinose.

The pentosans of cottonseed meal are not soluble in diastase, and are contained entirely in the nitrogen-free extract, unless an unusually large quantity of hulls is present.

Cottonseed meal contains no starch.

No appreciable quantity of sucrose or reducing sugars is present.

Small quantities of organic acids are present, the average of five samples being 0.48 per cent.